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TITLE: Uncarboxylated Osteocalcin and Gprc6a Axis Produce Intratumoral Androgens in Castration-Resistant Prostate Cancer

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14. ABSTRACT Castrate resistant prostate cancer (CRPC) represents the final and lethal disease state in the progression of prostate cancer. CRPC patients often develop bone metastasis resulting in bone fractures and morbidity. Recently, tumor cells have been shown to activate androgen receptor signaling via multiple pathways, despite castrate levels of testosterone. One such adaptive mechanism is the "intracrine" production of androgens in the primary tumor and/or at metastatic sites by the activity of androgen biosynthetic enzymes. Recent study shows that Gprc6a/Osteocalcin axis regulates physiological androgen biosynthesis in testis. Since Osteocalcin is overexpressed in patients with bone metastasis and existence of intratumoral androgen synthesis in bone metastasis, we hypothesized that bone tumor expressed Osteocalcin can induce intratumoral androgen synthesis in bone metastasis. We show that in VCaP model system Gprc6a is expressed and overexpression of its ligand Osteocalcin in these cells leads to expression of androgen biosynthetic enzymes. This data suggest that prostate cancer bone tumors hijack Osteocalcin/Gprc6a axis for the production of intratumoral androgens via overexpression of certain androgen biosynthetic enzyme expression. Bone tumor expressed androgens promote disease progression via tumoral androgen production and androgen receptor activation.					
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1. INTRODUCTION:

Castrate resistant prostate cancer (CRPC) represents the final and lethal disease state in the progression of prostate cancer. Recently, tumor cells have been shown to activate androgen receptor signaling via multiple pathways, despite castrate levels of testosterone. One such adaptive mechanism is the “intracrine” production of androgens in the primary tumor and/or at metastatic sites by the activity of androgen biosynthetic enzymes(1).

CRPC is often characterized by disease progression in bone, and osteoblasts are known to express osteocalcin. Osteocalcin undergoes carboxylation at multiple glutamic acids, the resultant gamma carboxylated Osteocalcin interacts with bone extracellular matrix associated calcium and hydroxyapatite and deposited in the bone matrix. Some Osteocalcin is released into circulation without undergoing decarboxylation and recently Karsenty's group showed that circulating uncarboxylated Osteocalcin is a potent factor inducing androgen production in Leydig cells in testes (2). Osteocalcin binds and activates a novel cell surface receptor Gprc6a in Leydig cells to induce androgen biosynthetic enzyme expression and androgen production. However, osteocalcin is dysregulated in CRPC, with higher levels of the uncarboxylated form found in patients with bone metastasis (3). We thus hypothesize that uncarboxylated osteocalcin, expressed by osteoblasts or tumor cells, leads to local biosynthesis of androgens, thereby contributing to expansion of the metastatic deposit in bone, despite castrate levels of serum testosterone. Thus, just as the skeleton regulates fertility in an endocrine fashion, and it may also promote bone metastasis via an “intracrine” mechanism.

2. KEYWORDS:

CRPC:	Castrate resistant prostate cancer
PC:	Prostate Cancer
Gprc6a	A seven transmembrane G-protein coupled receptor
AKR1C3:	Aldo Keto Reductase 1C3
17BHSD	17 beta Hydroxysteroid Dehydrogenase
RTPCR	Real time Polymerase Chain Reaction
SCID	Severe combined immuno deficient
VCaP	Vertebral metastasis prostate cancer cell line
DHT	Dihydroxy testosterone.
RFP	Red fluorescence protein

3. OVERALL PROJECT SUMMARY:

Major Goals:

Goal 1: Demonstrate the functional Gprc6a expression in prostate cancer cells

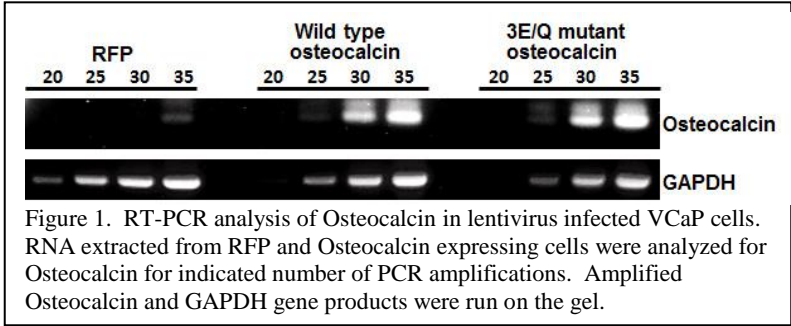
Goal 2: Determine the clinical significance of osteocalcin, Gprc6a, and androgen biosynthetic enzymes during CRPC progression.

Major activities:

1. We generated stable VCaP cell lines expressing RFP (red fluorescence protein as control) Osteocalcin and mutant Osteocalcin using lentivirus mediated stable infections.
2. Determined the gene expression of Gprc6a and androgen biosynthetic gene expression in Osteocalcin and mutant Osteocalcin infected cells.

Specific objectives:

Expression of Osteocalcin forms in VCaP cells: We used a lentiviral system for expressing Osteocalcin and mutated Osteocalcin. Osteocalcin is mutated at three positions where glutamic acid residue at 17, 18 and 24 were mutated to glutamine to prevent carboxylation. Both native and mutated osteocalcin was cloned into lentiviral expression plasmids. Viral stocks were infected with VCaP cells to express RFP, Osteocalcin and mutated Osteocalcin. Osteocalcin gene expression was determined using RT-PCR method. Osteocalcin and



mutated Osteocalcin was expressed in VCaP cells (Figure 1). Conditioned media was collected from RFP, osteocalcin and mutated Osteocalcin expressing cells. ELISA quatitiation show that Osteocalcin is secreted from the VCaP-osteocalcin cells (Figure 2). ELISA does not detect mutated Osteocalcin in mutated osteocacin expressing cells.

Androgen biosynthetic gene expression in VCaP cells: To determine the functional Gprc6a expression in VCaP cells, androgen biosynthetic enzyme expression was determined in VCaP RFP, wild type Osteocalcin and 3E/Q mutant Osteocalcin infected cells. VCaP and VCaP transfectants express Gprc6a. HSD3B2 and HSD17B6 expression was increased in both wild type and 3E/Q mutant Osteocalcin infected cells (Figure 3).

4. KEY RESEARCH ACCOMPLISHMENTS:

We demonstrate that Osteocalcin overexpressing cells induce androgen biosynthetic enzyme expression in prostate cancer cells. Here we show that HSD17B6 and HSD3B2 protein expression is increased in Osteocalcin and mutated Osteocalcin overexpressing cells compared to RFP expressing VCaP cells.

5. CONCLUSIONS:

Our data show that Osteocalcin/Gprc6a axis is functional in VCaP prostate cancer cells. This pathway can induce intra tumoral androgen synthesis in prostate cancer bone metastasis and drive androgen receptor mediated bone tumor growth. Ongoing experiments in determining the role of Osteocalcin in bone tumor growth and androgen production, and determining the expression of Osteocalcin, Gprc6a and androgen bioisynthetic enzyme expression in human CRPC tissue may provide significance of this pathway in the promotion of bone metastasis in prostate cancer patients.

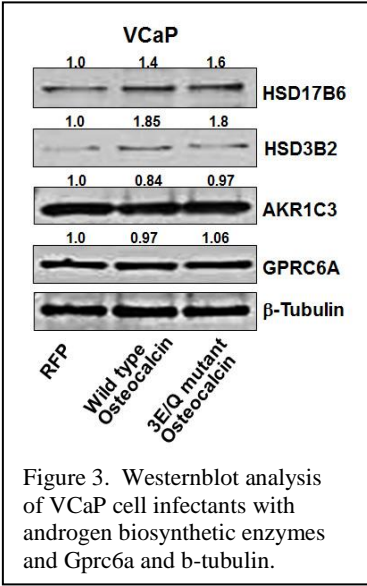
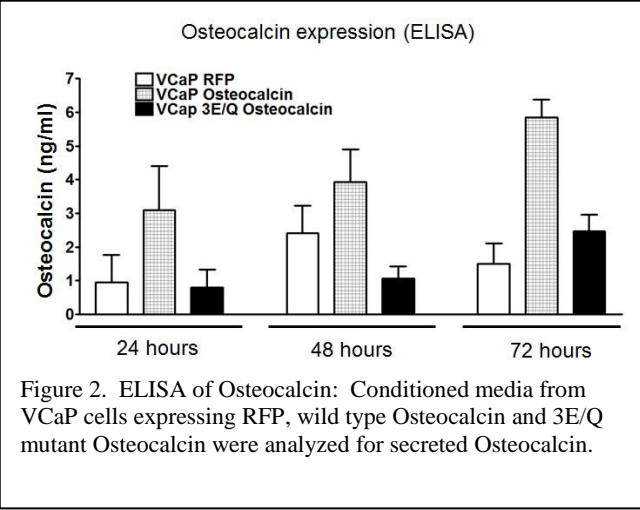
6. PUBLICATIONS, ABSRACTS, AND PRESENTATIONS:

None.

7. INVENTIONS, PATENTS AND LICENCES:

None.

8. REPORTABLE OUTCOMES:



None.

9. OTHER ACHIEVEMENT:

We developed three stable cells lines through lentivirus mediated expression of RFP, Osteocalcin and 3E/Q mutated Osteocalcin in VCaP cells.

10. REFERENCES:

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11. APPENDICES:

None.